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Attorney Docket No.:

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
APPLICATION FEE TRANSMITTAL

Box Patent Application  
Hon. Commissioner of Patents and Trademarks  
Washington, DC 20231

Sir:

Transmitted herewith for filing is the patent application of

Applicant(s):

Title:

22 pages of specification 0 sheets of drawings

2 sheets of Declaration and Power of Attorney

[x] The filing fee is calculated as follows:

Basic Fee: \$770.00

Total Claims:  $35 - 20 = 15 \times 22 =$  \$330.00

Independent Claims:  $1 - 3 = 0 \times 80 =$  \$ 0

Total Fee: \$1100.00

[x] Priority of application serial nos. 0931/96 filed August 30, 1996 in Denmark and 1259/96 filed November 8, 1996 in Denmark are claimed under 35 U.S.C. 119.

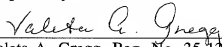
**Certified copies will follow.**

[x] The benefit of application serial nos. 60/035,905 filed on January 24, 1997 and 60/036,226 filed on January 24, 1997 in the U.S. are claimed under 35 U.S.C. 120.

[x] Please charge the required fee, estimated to be \$1100.00, to Novo Nordisk of North America, Inc., Deposit Account No. 14-1447. A duplicate of this sheet is enclosed.

Respectfully submitted,

Date: September 2, 1997

  
\_\_\_\_\_  
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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of: Knudsen et al.

Serial No.: To Be Assigned

Group Art Unit: To Be Assigned

Filed: September 2, 1997

Examiner: To Be Assigned

For: GLP-2 Derivatives

**PRELIMINARY AMENDMENT**

Hon. Commissioner of Patents and Trademarks  
Washington, DC 20231

Sir:

Before the above-captioned application is taken up for examination, entry of the following amendment is respectfully requested:

**IN THE SPECIFICATION:**

At page 1, before the first line, insert

**--CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims priority under 35 USC 120 to USSN 60/035,905, filed 24 January 1997, and USSN 60/036,226, filed 24 January 1997, and under 35 USC 119 of Danish applications 0931/96 filed 30 August 1996, and 1259/96 filed 8 November 1996, the contents of which applications are fully incorporated herein by reference.--

**IN THE CLAIMS:**

Please cancel claims 35-38 and 40, and amend the claims as follows:

Please amend claim 3 as follows:

At line 1, delete "or 2".

Please amend claim 4 as follows:

At line 1, delete "anyone of the preceding claims" and insert --claim 1--.

Please amend claim 5 as follows:

At line 1, delete "anyone of the claims 1-3" and insert --claim 1--.

Please amend claim 6 as follows:

At line 1, delete "anyone of the preceding claims" and insert --claim 1--.

Please amend claim 13 as follows:

At line 1, delete "anyone of the preceding claims" and insert --claim 1--.

Please amend claim 14 as follows:

At line 1, delete "any of claims 1-12" and insert --claim 1--.

Please amend claim 15 as follows:

At line 1, delete "any of claims 1-12" and insert --claim 1--.

Please amend claim 17 as follows:

At line 1, delete "any of claims 1-12" and insert --claim 1--.

Please amend claim 19 as follows:

At line 1, delete "any of claims 1-12" and insert --claim 1--.

Please amend claim 20 as follows:

At line 1, delete "any of claims 1-12" and insert --claim 1--.

Please amend claim 21 as follows:

At line 1, delete "any of claims 1-12" and insert --claim 1--.

Please amend claim 22 as follows:

At line 1, delete "any of claims 1-12" and insert --claim 1--.

Please amend claim 23 as follows:

At line 1, delete "any of claims 1-12" and insert --claim 1--.

Please amend claim 24 as follows:

At line 1, delete "any of claims 1-12" and insert --claim 1--.

Please amend claim 25 as follows:

At line 1, delete "any of claims 1-12" and insert --claim 1--.

Please amend claim 26 as follows:

At line 1, delete "any of claims 1-12" and insert --claim 1--.

Please amend claim 27 as follows:

At line 1, delete "any of the preceding claims" and insert --claim 1--.

Please amend claim 28 as follows:

At line 1, delete "any one of claims 1-26" and insert --claim 1--.

Please amend claim 29 as follows:

At line 1, delete "any one of the preceding claims" and insert --claim 1--.

Please amend claim 31 as follows:

At line 1, delete "any of the claims 29 and 30" and insert --claim 29--.

Please amend claim 32 as follows:

At line 1, delete "any of the preceding claims" and insert --claim 1--.

Please amend claim 33 as follows:

At line 1, delete "anyone of the preceding claims" and insert --claim 1--.

Please amend claim 34 as follows:

At line 1-2, delete "any of the preceding claims" and insert --claim 1--.

Please amend claim 39 as follows:


At line 3, delete "any one of the claims 1-33" and insert --claim 1--.

#### REMARKS

This amendment is submitted to correct improper multiple dependent claims. Since only dependencies are altered, there is no new matter added, and entry of the amendment is respectfully requested.

Respectfully submitted,

Date: 2 September 1997

  
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## GLP-2 DERIVATIVES

### FIELD OF THE INVENTION

- 5 The present invention relates to novel derivatives of human glucagon-like peptide-2 (hGLP-2) and analogues thereof and fragments thereof and analogues of such fragments which have a protracted profile of action and to methods of making and using them.

### 10 BACKGROUND OF THE INVENTION

- Peptides are widely used in medical practice, and since they can be produced by recombinant DNA technology it can be expected that their importance will increase also in the years to come. When native peptides or analogues thereof are used in therapy it is
- 15 generally found that they have a high clearance. A high clearance of a therapeutic agent is inconvenient in cases where it is desired to maintain a high blood level thereof over a prolonged period of time since repeated administrations will then be necessary. Examples of peptides which have a high clearance are: ACTH, corticotropin-releasing factor, angiotensin, calcitonin, insulin, glucagon, glucagon-like peptide-1, glucagon-like peptide-2, insulin-like
- 20 growth factor-1, insulin-like growth factor-2, gastric inhibitory peptide, growth hormone-releasing factor, pituitary adenylate cyclase activating peptide, secretin, enterogastrin, somatostatin, somatotropin, somatomedin, parathyroid hormone, thrombopoietin, erythropoietin, hypothalamic releasing factors, prolactin, thyroid stimulating hormones, endorphins, enkephalins, vasopressin, oxytocin, opiods and analogues thereof, superoxide
- 25 dismutase, interferon, asparaginase, arginase, arginine deaminase, adenosine deaminase and ribonuclease. In some cases it is possible to influence the release profile of peptides by applying suitable pharmaceutical compositions, but this approach has various shortcomings and is not generally applicable.
- 30 The amino acid sequence of GLP-2 and other preproglucagon fragments is given *i.a.* by Schmidt *et al.* (*Diabetologia* 28 704-707 (1985)). Little is known about the physical chemical properties of GLP-2 but GLP-2 is expected, like GLP-1, to be a highly flexible and unstable molecule. GLP-2 and fragments thereof and analogues of GLP-2 and fragments thereof are

potentially useful *i.e.* in regulation of appetite and in the treatment of small bowel syndrome. However, the high clearance limits the usefulness of these compounds, and thus there still is a need for improvements in this field.

5

## SUMMARY OF THE INVENTION

- Preproglucagon, from which GLP-2 originates, is synthesized *i.e.* in the L-cells in the distal ileum, in the pancreas and in the brain. Processing of preproglucagon to give GLP-1 and
- 10 GLP-2 occurs mainly in the L-cells. GLP-2 is a 34 amino acid residue peptide. A simple system is used to describe fragments, analogues and derivatives of GLP-2. Thus, for example, Lys<sup>20</sup>GLP-2(1-33) designates a fragment of GLP-2 formally derived from GLP-2 by deleting the amino acid residues No. 34 and substituting the naturally occurring amino acid residue in position 20 (Arg) by Lys. Similarly, Arg<sup>30</sup>Lys<sup>35</sup>(N<sup>6</sup>-tetradecanoyl)GLP-1(1-35)
- 15 designates a derivative of a GLP-2 analogue formally derived from GLP-2 by C-terminal addition of a Lys residue, exchange of the naturally occurring amino acid residue in position 30 (Lys) with an Arg residue and tetradecanoylation of the  $\epsilon$ -amino group of the Lys residue in position 35.
- 20 In its broadest aspect, the present invention relates to derivatives of GLP-2 and analogues thereof. The derivatives according to the invention have interesting pharmacological properties, in particular they have a more protracted profile of action than the parent peptides.
- 25 In the present text, unless otherwise specified, "GLP-2" designates human GLP-2. The designation "an analogue" is used to designate a peptide wherein one or more amino acid residues of the parent peptide have been substituted by another amino acid residue and/or wherein one or more amino acid residues of the parent peptide have been deleted and/or wherein one or more amino acid residues have been added to the parent peptide.
- 30 The term "derivative" is used in the present text to designate a peptide in which one or more of the amino acid residues have been chemically modified, *e.g.* by alkylation, acylation, ester formation or amide formation.

The term "a GLP derivative" is used in the present text to designate a derivative of GLP-2 or an analogue thereof. In the present text, the parent peptide from which such a derivative is formally derived is in some places referred to as the "GLP moiety" of the derivative.

5

In a preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent attached to any one amino acid residue.

In another preferred embodiment, the present invention relates to a GLP-2 derivative having  
10 a lipophilic substituent attached to any one amino acid residue with the proviso that only if the substituent has an  $\omega$ -carboxylic acid group or is an alkyl group can it be attached to the N-terminal or C-terminal amino acid residue of the parent peptide.

In another preferred embodiment, the present invention relates to a GLP-2 derivative  
15 wherein the lipophilic substituent comprises from 4 to 40 carbon atoms, more preferred from 8 to 25 carbon atoms.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to an amino acid residue in such a way that a  
20 carboxyl group of the lipophilic substituent forms an amide bond with an amino group of the amino acid residue.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to an amino acid residue in such a way that an  
25 amino group of the lipophilic substituent forms an amide bond with a carboxyl group of the amino acid residue.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer.

30

In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an unbranched alkane  $\alpha,\omega$ -dicarboxylic acid group having from 1 to 7 methylene groups,



preferably two methylene groups which spacer forms a bridge between an amino group of the parent peptide and an amino group of the lipophilic substituent.

- In a further preferred embodiment, the present invention relates to a GLP-2 derivative  
 5 wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or a dipeptide such as Gly-Lys. In the present text, the expression "a dipeptide such as Gly-Lys" is used to designate a dipeptide wherein the C-terminal amino acid residue is Lys, His or Trp, preferably Lys, and wherein the N-terminal amino acid residue is selected from the group comprising Ala, Arg, Asp, Asn, Gly, Glu, Gln,  
 10 Ile, Leu, Val, Phe and Pro.

- In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys and wherein a  
 15 carboxyl group of the parent peptide forms an amide bond with an amino group of a Lys residue or a dipeptide containing a Lys residue, and the other amino group of the Lys residue or a dipeptide containing a Lys residue forms an amide bond with a carboxyl group of the lipophilic substituent.
- 20 In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys and wherein an amino group of the parent peptide forms an amide bond with a carboxylic group of the amino acid or dipeptide spacer, and an amino group of the amino acid or dipeptide spacer forms an  
 25 amide bond with a carboxyl group of the lipophilic substituent.

- In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys and wherein a  
 30 carboxyl group of the parent peptide forms an amide bond with an amino group of the amino acid or dipeptide spacer, and the carboxyl group of the amino acid or dipeptide spacer forms an amide bond with an amino group of the lipophilic substituent.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys, and wherein a carboxyl group of the parent peptide forms an amide bond with an amino group of Asp or Glu, or a dipeptide containing an Asp or Glu residue, and a carboxyl group of the spacer forms an amide bond with an amino group of the lipophilic substituent.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which comprises a partially or completely hydrogenated cyclopentano-phenanthrene skeleton.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is a straight-chain or branched alkyl group.

15 In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is the acyl group of a straight-chain or branched fatty acid.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is an acyl group selected from the group comprising  
 20  $\text{CH}_3(\text{CH}_2)_n\text{CO}-$ , wherein  $n$  is 4 to 38, preferably  $\text{CH}_3(\text{CH}_2)_6\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_8\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_{10}\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_{14}\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_{16}\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_{18}\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_{20}\text{CO}-$  and  $\text{CH}_3(\text{CH}_2)_{22}\text{CO}-$ .

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having  
 25 a lipophilic substituent which is an acyl group of a straight-chain or branched alkane  $\alpha,\omega$ -dicarboxylic acid.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is an acyl group selected from the group comprising  
 30  $\text{HOOC}(\text{CH}_2)_m\text{CO}-$ , wherein  $m$  is 4 to 38, preferably  $\text{HOOC}(\text{CH}_2)_{14}\text{CO}-$ ,  $\text{HOOC}(\text{CH}_2)_{16}\text{CO}-$ ,  $\text{HOOC}(\text{CH}_2)_{18}\text{CO}-$ ,  $\text{HOOC}(\text{CH}_2)_{20}\text{CO}-$  and  $\text{HOOC}(\text{CH}_2)_{22}\text{CO}-$ .

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having

a lipophilic substituent which is a group of the formula  $\text{CH}_3(\text{CH}_2)_p((\text{CH}_2)_q\text{COOH})\text{CHNH}-\text{CO}(\text{CH}_2)_2\text{CO}-$ , wherein p and q are integers and p+q is an integer of from 8 to 40, preferably from 12 to 35.

- 5 In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is a group of the formula  $\text{CH}_3(\text{CH}_2)_r\text{CO}-\text{NHCH}(\text{COOH})(\text{CH}_2)_2\text{CO}-$ , wherein r is an integer of from 10 to 24.

- 10 In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is a group of the formula  $\text{CH}_3(\text{CH}_2)_s\text{CO}-\text{NHCH}((\text{CH}_2)_2\text{COOH})\text{CO}-$ , wherein s is an integer of from 8 to 24.

- In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is a group of the formula  $\text{COOH}(\text{CH}_2)_t\text{CO}-$  wherein t is an integer of from 8 to 24.

- 15 In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is a group of the formula  $-\text{NHCH}(\text{COOH})(\text{CH}_2)_u\text{NH}-\text{CO}(\text{CH}_2)_v\text{CH}_3$ , wherein u is an integer of from 8 to 18.

- 20 In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is a group of the formula  $-\text{NHCH}(\text{COOH})(\text{CH}_2)_w\text{NH}-\text{COCH}(\text{CH}_2)_2\text{COOH})\text{NH}-\text{CO}(\text{CH}_2)_x\text{CH}_3$ , wherein w is an integer of from 10 to 16.

- 25 In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is a group of the formula  $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH}-\text{CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NH}-\text{CO}(\text{CH}_2)_x\text{CH}_3$ , wherein x is an integer of from 10 to 16.

- In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is a group of the formula  $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH}-\text{CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NHCO}(\text{CH}_2)_y\text{CH}_3$ , wherein y is zero or an integer of from 1 to 22.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative which has one lipophilic substituent.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative which has two lipophilic substituents.

- 5 In a further preferred embodiment, the present invention relates to a GLP-2 derivative in which the C-terminal amino acid residue is present in the form of the amide.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which can be negatively charged.

10

In a further preferred embodiment, the present invention relates to a GLP-2 derivative the parent peptide of which is selected from the group comprising GLP-2(1-35) or an analogue thereof.

- 15 In a further preferred embodiment, the present invention relates to a GLP-2 derivative derived from a GLP-2 fragment selected from the group comprising GLP-2(1-30); GLP-2(1-31); GLP-2(1-32); GLP-2(1-33); GLP-2(1-34) and GLP-2(1-35).

- In a further preferred embodiment, the present invention relates to a GLP-2 derivative  
20 wherein the designation analogue comprises derivatives wherein a total of up to ten amino acid residues have been exchanged with any  $\alpha$ -amino acid residue.

- In a further preferred embodiment, the present invention relates to a derivative of a GLP-2 analogue wherein the designation analogue implies that the parent peptide is human GLP-2  
25 wherein a total of up to six, more preferred up to three, amino acid residues have been added, deleted or substituted with other amino acid residues which can be coded for by the genetic code.

- In a further preferred embodiment, the present invention relates to a GLP-2 derivative  
30 wherein the parent peptide is selected from the group comprising Lys<sup>20</sup>GLP-2(1-33) and Lys<sup>20</sup>Arg<sup>30</sup>GLP-2(1-33).

In a further preferred embodiment, the present invention relates to a GLP-2 derivative

wherein the parent peptide is Arg<sup>30</sup>Lys<sup>34</sup>GLP-2(1-34).

- In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein the parent peptide is selected from the group comprising Arg<sup>30</sup>Lys<sup>35</sup>GLP-2(1-35);
- 5 Arg<sup>30,35</sup>Lys<sup>20</sup>GLP-2(1-35) and Arg<sup>35</sup>GLP-2(1-35).

In a further preferred embodiment, the present invention relates to a GLP-2 derivative which is selected from the group comprising

- 10 Lys<sup>20</sup>(N<sup>ε</sup>-tetradecanoyl)GLP-2(1-33);  
 Lys<sup>20,30</sup>-bis(N<sup>ε</sup>-tetradecanoyl)GLP-2(1-33);  
 Lys<sup>20</sup>(N<sup>ε</sup>-tetradecanoyl)Arg<sup>30</sup>GLP-2(1-33);  
 Arg<sup>30</sup>Lys<sup>35</sup>(N<sup>ε</sup>-tetradecanoyl)GLP-2(1-35);  
 Arg<sup>30,35</sup>Lys<sup>20</sup>(N<sup>ε</sup>-tetradecanoyl)GLP-2(1-35);
- 15 Arg<sup>25</sup>Lys<sup>30</sup>(N<sup>ε</sup>-tetradecanoyl)GLP-2(1-35);  
 Arg<sup>30</sup>Lys<sup>34</sup>(N<sup>ε</sup>-tetradecanoyl)GLP-2(1-34);  
 Lys<sup>20</sup>(N<sup>ε</sup>-(ω-carboxynonadecanoyl))GLP-2(1-33);  
 Lys<sup>20,30</sup>-bis(N<sup>ε</sup>-(ω-carboxynonadecanoyl))GLP-2(1-33);  
 Lys<sup>20</sup>(N<sup>ε</sup>-(ω-carboxynonadecanoyl))Arg<sup>30</sup>GLP-2(1-33);
- 20 Arg<sup>30</sup>Lys<sup>35</sup>(N<sup>ε</sup>-(ω-carboxynonadecanoyl))GLP-2(1-35);  
 Arg<sup>30,35</sup>Lys<sup>20</sup>(N<sup>ε</sup>-(ω-carboxynonadecanoyl))GLP-2(1-35);  
 Arg<sup>25</sup>Lys<sup>30</sup>(N<sup>ε</sup>-(ω-carboxynonadecanoyl))GLP-2(1-35); and  
 Arg<sup>30</sup>Lys<sup>34</sup>(N<sup>ε</sup>-(ω-carboxynonadecanoyl))GLP-2(1-34).
- 25 In a further preferred embodiment, the present invention relates to a pharmaceutical composition comprising a GLP-2 derivative and a pharmaceutically acceptable vehicle or carrier.
- In a further preferred embodiment, the present invention relates to the use of a GLP-2 derivative according to the invention for the preparation of a medicament which has a more protracted action than the parent peptide.
- 30

In a further preferred embodiment, the present invention relates to the use of a GLP-2 derivative according to the invention for the preparation of a medicament with protracted effect for the treatment of obesity.

- 5 In a further preferred embodiment, the present invention relates to the use of a GLP-2 derivative according to the invention for the preparation of a medicament with protracted effect for the treatment of small bowel syndrome.

## 10 DETAILED DESCRIPTION OF THE INVENTION

- To obtain a satisfactory protracted profile of action of the GLP-2 derivative, the lipophilic substituent attached to the GLP-2 moiety preferably comprises 4-40 carbon atoms, in particular 8-25 carbon atoms. The lipophilic substituent may be attached to an amino group
- 15 of the GLP-2 moiety by means of a carboxyl group of the lipophilic substituent which forms an amide bond with an amino group of the amino acid to which it is attached. As an alternative, the lipophilic substituent may be attached to said amino acid in such a way that an amino group of the lipophilic substituent forms an amide bond with a carboxyl group of the amino acid. As a further option, the lipophilic substituent may be linked to the GLP-2 moiety
- 20 via an ester bond. Formally, the ester can be formed either by reaction between a carboxyl group of the GLP-2 moiety and a hydroxyl group of the substituent-to-be or by reaction between a hydroxyl group of the GLP-2 moiety and a carboxyl group of the substituent-to-be. As a further alternative, the lipophilic substituent can be an alkyl group which is introduced into a primary amino group of the GLP-2 moiety.

25

- In one preferred embodiment of the invention, the lipophilic substituent is attached to the GLP-2 moiety by means of a spacer in such a way that a carboxyl group of the spacer forms an amide bond with an amino group of the GLP-2 moiety. Examples of suitable spacers are succinic acid, Lys, Glu or Asp, or a dipeptide such as Gly-Lys. When the spacer is succinic
- 30 acid, one carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the other carboxyl group thereof may form an amide bond with an amino group of the lipophilic substituent. When the spacer is Lys, Glu or Asp, the carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the

- amino group thereof may form an amide bond with a carboxyl group of the lipophilic substituent. When Lys is used as the spacer, a further spacer may in some instances be inserted between the  $\epsilon$ -amino group of Lys and the lipophilic substituent. In one preferred embodiment, such a further spacer is succinic acid which forms an amide bond with the  $\epsilon$ -
- 5 amino group of Lys and with an amino group present in the lipophilic substituent. In another preferred embodiment such a further spacer is Glu or Asp which forms an amide bond with the  $\epsilon$ -amino group of Lys and another amide bond with a carboxyl group present in the lipophilic substituent, that is, the lipophilic substituent is a N<sup>ε</sup>-acylated lysine residue.
- 10 In another preferred embodiment of the present invention, the lipophilic substituent has a group which can be negatively charged. One preferred group which can be negatively charged is a carboxylic acid group.
- The parent peptide can be produced by a method which comprises culturing a host cell
- 15 containing a DNA sequence encoding the peptide and capable of expressing the peptide in a suitable nutrient medium under conditions permitting the expression of the peptide, after which the resulting peptide is recovered from the culture.
- The medium used to culture the cells may be any conventional medium suitable for growing
- 20 the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection). The peptide produced by the cells may then be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration,
- 25 precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. ion exchange chromatography, gel filtration chromatography, affinity chromatography, or the like, dependent on the type of peptide in question.
- 30 The DNA sequence encoding the parent peptide may suitably be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the peptide by hybridisation using synthetic oligonucleotide probes in accordance with standard techniques (see, for example,

Sambrook, J, Fritsch, EF and Maniatis, T, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, 1989). The DNA sequence encoding the peptide may also be prepared synthetically by established standard methods, e.g. the phosphoramidite method described by Beaucage and Caruthers, *Tetrahedron Letters* **22** (1981), 1859 - 1869, or the method described by Matthes *et al.*, *EMBO Journal* **3** (1984), 801 - 805. The DNA sequence may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202 or Saiki *et al.*, *Science* **239** (1988), 487 - 491.

- 10 The DNA sequence may be inserted into any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when
- 15 introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

The vector is preferably an expression vector in which the DNA sequence encoding the peptide is operably linked to additional segments required for transcription of the DNA, such

20 as a promoter. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA encoding the peptide of the invention in a variety of host cells are well known in the art, cf. for instance Sambrook *et al.*, *supra*.

25 The DNA sequence encoding the peptide may also, if necessary, be operably connected to a suitable terminator, polyadenylation signals, transcriptional enhancer sequences, and translational enhancer sequences. The recombinant vector of the invention may further comprise a DNA sequence enabling the vector to replicate in the host cell in question.

30 The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell or one which confers resistance to a drug, e.g. ampicillin, kanamycin, tetracyclin, chloramphenicol, neomycin, hygromycin or methotrexate.



- To direct a parent peptide of the present invention into the secretory pathway of the host cells, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) may be provided in the recombinant vector. The secretory signal sequence is joined to the DNA sequence encoding the peptide in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the peptide. The secretory signal sequence may be that normally associated with the peptide or may be from a gene encoding another secreted protein.
- 10 The procedures used to ligate the DNA sequences coding for the present peptide, the promoter and optionally the terminator and/or secretory signal sequence, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (cf., for instance, Sambrook *et al.*, *supra*).
- 15 The host cell into which the DNA sequence or the recombinant vector is introduced may be any cell which is capable of producing the present peptide and includes bacteria, yeast, fungi and higher eukaryotic cells. Examples of suitable host cells well known and used in the art are, without limitation, *E. coli*, *Saccharomyces cerevisiae*, or mammalian BHK or CHO cell lines.
- 20 The GLP-2 derivatives of the invention can be prepared by introducing the lipophilic substituent into the parent GLP-2 or GLP-2 analogue using methods known *per se*, see for example WO 95/07931, the contents of which is hereby incorporated in its entirety by reference.
- 25 N<sup>ε</sup>-acylation of a Lys residue can be carried out by using an activated amide of the acyl group to be introduced as the acylating agent, e.g. the amide with benzotriazole. The acylation is carried out in a polar solvent in the presence of a base.

30

### Pharmaceutical compositions

Pharmaceutical compositions containing a GLP-2 derivative according to the present

invention may be administered parenterally to patients in need of such a treatment. Parenteral administration may be performed by subcutaneous, intramuscular or intravenous injection by means of a syringe, optionally a pen-like syringe. Alternatively, parenteral administration can be performed by means of an infusion pump. A further option is a composition which may be a powder or a liquid for the administration of the GLP-2 derivative in the form of a nasal or pulmonal spray. As a still further option, the GLP-2 derivatives of the invention can also be administered transdermally, e.g. from a patch, optionally a iontophoretic patch, or transmucosally, e.g. buccally.

- 10 Pharmaceutical compositions containing a GLP-2 derivative of the present invention may be prepared by conventional techniques, e.g. as described in Remington's Pharmaceutical Sciences, 1985 or in Remington: *The Science and Practice of Pharmacy*, 19<sup>th</sup> edition, 1995.

- Thus, the injectable compositions of the GLP-2 derivative of the invention can be prepared using the conventional techniques of the pharmaceutical industry which involves dissolving and mixing the ingredients as appropriate to give the desired end product.

- Thus, according to one procedure, the GLP-2 derivative is dissolved in an amount of water which is somewhat less than the final volume of the composition to be prepared. An isotonic agent, a preservative and a buffer is added as required and the pH value of the solution is adjusted - if necessary - using an acid, e.g. hydrochloric acid, or a base, e.g. aqueous sodium hydroxide as needed. Finally, the volume of the solution is adjusted with water to give the desired concentration of the ingredients.

- 25 Examples of isotonic agents are sodium chloride, mannitol and glycerol.

Examples of preservatives are phenol, m-cresol, methyl p-hydroxybenzoate and benzyl alcohol.

- 30 Examples of suitable buffers are sodium acetate and sodium phosphate.

Further to the above-mentioned components, solutions containing a GLP-2 derivative according to the present invention may also contain a surfactant in order to improve the

solubility and/or the stability of the derivative.

A composition for nasal administration of GLP-2 may, for example, be prepared as described in European Patent No. 272097 (to Novo Nordisk A/S) or in WO 93/18785.

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The GLP-2 derivatives of this invention can be used in the treatment of various diseases. The particular GLP-2 derivative to be used and the optimal dose level for any patient will depend on the disease to be treated and on a variety of factors including the efficacy of the specific peptide derivative employed, the age, body weight, physical activity, and diet of the patient, on a possible combination with other drugs, and on the severity of the case. It is recommended that the dosage of the GLP-2 derivative of this invention be determined for each individual patient by those skilled in the art in a similar way as for known parent peptides.

- 10 15 The pharmacological properties of the compounds of the invention can be tested e.g. as described in our International Patent Application No. PCT/DK97/00086 the contents of which is hereby incorporated in its entirety by reference.

- 20 The present invention is further illustrated by the following examples which, however, are not to be construed as limiting the scope of protection. The features disclosed in the foregoing description and in the following examples may, both separately and in any combination thereof, be material for realizing the invention in diverse forms thereof.

25

## EXAMPLES

The following acronyms for commercially available chemicals are used:

- |           |  |
|-----------|--|
| NMP :     | N-Methyl-2-pyrrolidone.                            |
| 30 EDPA : | N-Ethyl-N,N-diisopropylamine.                      |
| TFA :     | Trifluoroacetic acid.                              |
| Myr-ONSu: | Tetradecanoic acid 2,5-dioxopyrrolidin-1-yl ester. |

**Abbreviations:**

PDMS: Plasma Desorption Mass Spectrometry

HPLC: High Performance Liquid Chromatography

5 amu: atomic mass units

**EXAMPLE 1**Synthesis of Lys<sup>36</sup> (N<sup>6</sup>-tetradecanoyl) hGLP-2.

10

A mixture of hGLP-2 (10.0 mg, 2.7  $\mu$ mol), EDPA (9.6 mg, 74.3  $\mu$ mol), NMP (210  $\mu$ l) and water (100  $\mu$ l) was gently shaken for 15 min. at room temperature. To the resulting mixture was added a solution of Myr-ONSu (21.5 mg, 6.6  $\mu$ mol) in NMP (32  $\mu$ l). The reaction mixture was gently shaken for 30 min. at room temperature, and an additional amount of a

15 solution of Myr-ONSu (14.4 mg, 4.4  $\mu$ mol) in NMP (22  $\mu$ l). The resulting mixture was gently shaken for 15 min. at room temperature. The reaction was quenched by the addition of a solution of glycine (4.5 mg, 4.5  $\mu$ mol) in 50% aqueous ethanol (451  $\mu$ l). The reaction mixture was purified by column chromatography using a cyanopropyl column (Zorbax 300SB-CN) and a standard acetonitrile/TFA system. The column was heated to 65°C and  
20 the acetonitrile gradient was 0-100% in 60 minutes. The title compound ( 5.0 mg, 47 %) was isolated from the eluate.

## CLAIMS

1. A GLP-2 derivative comprising a lipophilic substituent attached to any one amino acid residue.
- 5
2. A GLP-2 derivative according to claim 1 with the proviso that only if the substituent has an  $\omega$ -carboxylic acid group or is an alkyl group can it be attached to the N-terminal or C-terminal amino acid residue of the parent peptide.
- 10
3. A GLP-2 derivative according to claim 1 or 2, wherein the lipophilic substituent comprises from 4 to 40 carbon atoms, more preferred from 8 to 25.
4. A GLP-2 derivative according to anyone of the preceding claims, wherein said lipophilic substituent is attached to said amino acid in such a way that a carboxyl group of the
- 15
- lipophilic substituent forms an amide bond with an amino group of the amino acid.
5. A GLP-2 derivative according to anyone of the claims 1-3, wherein said lipophilic substituent is attached to said amino acid in such a way that an amino group of the lipophilic substituent forms an amide bond with a carboxyl group of the amino acid.
- 20
6. A GLP-2 derivative according to anyone of the preceding claims, wherein the lipophilic substituent is attached to the parent peptide by means of a spacer.
7. A GLP-2 derivative according to claim 6, wherein the spacer is an unbranched alkane
- 25
- $\alpha,\omega$ -dicarboxylic acid group having from 1 to 7 methylene groups, preferably two methylene groups which form a bridge between an amino group of the parent peptide and an amino group of the lipophilic substituent.
8. A GLP-2 derivative according to claim 6, wherein the spacer is an amino acid residue
- 30
- except Cys, or a dipeptide such as Gly-Lys.
9. A GLP-2 derivative according to claim 8, wherein a carboxyl group of the parent peptide forms an amide bond with an amino group of Lys or a dipeptide containing a Lys residue,

and the other amino group of the Lys or a dipeptide containing a Lys residue forms an amide bond with a carboxyl group of the lipophilic substituent.

- 10.A GLP-2 derivative according to claim 8, wherein an amino group of the parent peptide  
5 forms an amide bond with a carboxylic group of the amino acid or dipeptide spacer, and an amino group of the amino acid or dipeptide spacer forms an amide bond with a carboxyl group of the lipophilic substituent.
- 11.A GLP-2 derivative according to claim 8, wherein a carboxyl group of the parent peptide  
10 forms an amide bond with an amino group of the amino acid or dipeptide spacer, and the carboxyl group of the amino acid or dipeptide spacer forms an amide bond with an amino group of the lipophilic substituent.
- 12.A GLP-2 derivative according to claim 8, wherein a carboxyl group of the parent peptide  
15 forms an amide bond with an amino group of Asp or Glu, or a dipeptide containing an Asp or Glu residue, and a carboxyl group of the spacer forms an amide bond with an amino group of the lipophilic substituent.
- 13.A GLP-2 derivative according to anyone of the preceding claims, wherein the lipophilic  
20 substituent comprises a partially or completely hydrogenated cyclopentanophenathrene skeleton.
- 14.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is  
an straight-chain or branched alkyl group.
- 25 15.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is the acyl group of a straight-chain or branched fatty acid.
- 16.A GLP-2 derivative according to claim 15 wherein the acyl group is selected from the  
30 group comprising  $\text{CH}_3(\text{CH}_2)_n\text{CO}-$ , wherein  $n$  is 4 to 38, preferably  $\text{CH}_3(\text{CH}_2)_6\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_8\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_{10}\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_{14}\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_{16}\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_{18}\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_{20}\text{CO}-$  and  $\text{CH}_3(\text{CH}_2)_{22}\text{CO}-$ .

- 17.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is an acyl group of a straight-chain or branched alkane  $\alpha,\omega$ -dicarboxylic acid.
- 18.A GLP-2 derivative according to claim 17 wherein the acyl group is selected from the group comprising  $\text{HOOC}(\text{CH}_2)_m\text{CO}-$ , wherein m is 4 to 38, preferably  $\text{HOOC}(\text{CH}_2)_{14}\text{CO}-$ ,  $\text{HOOC}(\text{CH}_2)_{16}\text{CO}-$ ,  $\text{HOOC}(\text{CH}_2)_{18}\text{CO}-$ ,  $\text{HOOC}(\text{CH}_2)_{20}\text{CO}-$  and  $\text{HOOC}(\text{CH}_2)_{22}\text{CO}-$ .
- 19.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula  $\text{CH}_3(\text{CH}_2)_p((\text{CH}_2)_q\text{COOH})\text{CHNH-CO}(\text{CH}_2)_r\text{CO}-$ , wherein p and q are integers and p+q is an integer of from 8 to 40, preferably from 12 to 35.
- 20.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula  $\text{CH}_3(\text{CH}_2)_r\text{CO-NHCH}(\text{COOH})(\text{CH}_2)_s\text{CO}-$ , wherein r is an integer of from 10 to 24.
- 21.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula  $\text{CH}_3(\text{CH}_2)_s\text{CO-NHCH}((\text{CH}_2)_t\text{COOH})\text{CO}-$ , wherein s is an integer of from 8 to 24.
- 22.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula  $\text{COOH}(\text{CH}_2)_t\text{CO}-$  wherein t is an integer of from 8 to 24.
- 23.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula  $-\text{NHCH}(\text{COOH})(\text{CH}_2)_u\text{NH-CO}(\text{CH}_2)_v\text{CH}_3$ , wherein u is an integer of from 8 to 18.
- 24.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula  $-\text{NHCH}(\text{COOH})(\text{CH}_2)_w\text{NH-COCH}((\text{CH}_2)_x\text{COOH})\text{NH-CO}(\text{CH}_2)_y\text{CH}_3$ , wherein w is an integer of from 10 to 16.
- 25.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula  $-\text{NHCH}(\text{COOH})(\text{CH}_2)_z\text{NH-CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NH-CO}(\text{CH}_2)_x\text{CH}_3$ , wherein x is an integer of from 10 to 16.

26.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula  $-\text{NHCH}(\text{COOH})(\text{CH}_2)_x\text{NH-CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NHCO}(\text{CH}_2)_y\text{CH}_3$ , wherein y is zero or an integer of from 1 to 22.

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27.A GLP-2 derivative according to any of the preceding claims which has one lipophilic substituent.

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28.A GLP-2 derivative according to any one of claims 1-26 which has two lipophilic substituents.

15

29.A GLP-2 derivative according anyone of the preceding claims, wherein the parent peptide is selected from the group comprising GLP-2(1-30); GLP-2(1-31); GLP-2(1-32); GLP-2(1-33); GLP-2(1-34) and GLP-2(1-35) or an analogue or a fragment thereof.

20

30.A GLP-2 derivative according to claim 29, wherein the parent peptide is selected from the group comprising GLP-2(1-35) or an analogue or a fragment thereof.

31.A GLP-2 derivative according to any of the claims 29 and 30 wherein the designation analogue comprises derivatives wherein a total of up to ten amino acid residues have been exchanged with any  $\alpha$ -amino acid residue.

25

32.A GLP-2 derivative according to any of the preceding claims wherein the parent peptide is selected from the group comprising Lys<sup>20</sup>GLP-2(1-33); Lys<sup>20</sup>Arg<sup>30</sup>GLP-2(1-33); Arg<sup>30</sup>Lys<sup>36</sup>GLP-2(1-35); Arg<sup>30,36</sup>Lys<sup>20</sup>GLP-2(1-35); Arg<sup>36</sup>GLP-2(1-35); Arg<sup>30</sup>Lys<sup>34</sup>GLP-2(1-34).

30

33.A GLP-2 derivative according to anyone of the preceding claims, which is selected from the group consisting of

Lys<sup>20</sup>(N<sup>5</sup>-tetradecanoyl)GLP-2(1-33);

Lys<sup>20,30</sup>-bis(N<sup>5</sup>-tetradecanoyl)GLP-2(1-33);

Lys<sup>20</sup>(N<sup>5</sup>-tetradecanoyl)Arg<sup>30</sup>GLP-2(1-33);



- Arg<sup>20</sup>Lys<sup>35</sup>(N<sup>ε</sup>-tetradecanoyl)GLP-2(1-35);  
 Arg<sup>20,35</sup>Lys<sup>20</sup>(N<sup>ε</sup>-tetradecanoyl)GLP-2(1-35);  
 Arg<sup>35</sup>Lys<sup>30</sup>(N<sup>ε</sup>-tetradecanoyl)GLP-2(1-35);  
 Arg<sup>30</sup>Lys<sup>34</sup>(N<sup>ε</sup>-tetradecanoyl)GLP-2(1-34);  
 5 Lys<sup>20</sup>(N<sup>ε</sup>-(ω-carboxynonadecanoyl))GLP-2(1-33);  
 Lys<sup>20,36</sup>bis(N<sup>ε</sup>-(ω-carboxynonadecanoyl))GLP-2(1-33);  
 Lys<sup>20</sup>(N<sup>ε</sup>-(ω-carboxynonadecanoyl))Arg<sup>30</sup>GLP-2(1-33);  
 Arg<sup>30</sup>Lys<sup>35</sup>(N<sup>ε</sup>-(ω-carboxynonadecanoyl))GLP-2(1-35);  
 Arg<sup>30,35</sup>Lys<sup>20</sup>(N<sup>ε</sup>-(ω-carboxynonadecanoyl))GLP-2(1-35);  
 10 Arg<sup>35</sup>Lys<sup>30</sup>(N<sup>ε</sup>-(ω-carboxynonadecanoyl))GLP-2(1-35); and  
 Arg<sup>30</sup>Lys<sup>34</sup>(N<sup>ε</sup>-(ω-carboxynonadecanoyl))GLP-2(1-34).

- 34.A pharmaceutical composition comprising a GLP-2 derivative according to any of the preceding claims and a pharmaceutically acceptable vehicle or carrier.
- 15 35.Use of a GLP-2 derivative according to any of the claims 1-33 for the preparation of a medicament.
- 20 36.Use of a GLP-2 derivative according to any of the claims 1-33 for the preparation of a medicament with protracted effect.
- 37.Use of a GLP-2 derivative according to any of claims 1-33 for the preparation of a medicament with protracted effect for the treatment of obesity.
- 25 38.Use of a GLP-2 derivative according to any of claims 1-33 for the preparation of a medicament with protracted effect for the treatment of small bowel syndrome.
- 30 39.A method of treating obesity in a patient in need of such a treatment, comprising administering to the patient a therapeutically effective amount of a GLP-2 derivative according to any one of the claims 1-33 together with a pharmaceutically acceptable carrier.

5

**ABSTRACT**

5

10

**GLP-2 DERIVATIVES**

- 15 Derivatives of hGLP-2 and analogues thereof and fragments thereof and analogues of such fragments having a lipophilic substituent have interesting pharmacological properties, in particular they have a more protracted profile of action than the parent peptides.

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

the specification of which (check only one item below):

☐ is attached hereto

☒ was filed as United States application

Serial No. to be assigned

on September 2, 1997

and was amended

on \_\_\_\_\_

☐ was filed as PCT international application

Number \_\_\_\_\_

on \_\_\_\_\_

and was amended under PCT Article 19

on \_\_\_\_\_

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign applications(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign applications(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
Denmark	0931/96	August 30, 1996	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
Denmark	1259/96	November 8, 1996	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY**  
(Includes Reference to PCT International Applications)

Attorney's Docket Number:

5367.200-US

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this applications is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 1

Signature of Inventor 2

Signature of Inventor 3

Date

Date

Date